Schistosome-induced portacaval haemodynamic changes in *Rattus rattus* are associated with translocation of adult worms to the lungs

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SUMMARY

The presence of naturally portacaval shunts has been investigated in the vasculature of normal and *Schistosoma mansoni*-infected *Rattus rattus*. Using the technique of injecting Polystyrene microspheres in the superior mesenteric vein, we demonstrated that the presence of adult schistosomes in the lungs of *R. rattus* was not due to an innate anomaly of the rat vasculature but resulted from the formation of portacaval shunts during infection. In rats harbouring a bisexual infection, microspheres were only detected in the lungs from week 7. The development and increasing size of the shunts were maximal between weeks 7 and 10 and coincident with the translocation of adult worms from the portal tract to the lungs. At weeks 20–25, only 1–2% of the microspheres were recovered from the lungs, suggesting that the portacaval anastomoses have regressed due to reduction in portal hypertension after worm translocation. *R. rattus* with a male-only schistosome infection harboured adult worms in the lungs, indicating that the development of shunts does not solely depend upon egg deposition in the liver to generate hypertension. The relationships between the presence of the schistosomes in the lungs, the portacaval shunting and the resistance to reinfection is discussed.

Key words: *Schistosoma mansoni*, *Rattus rattus*, lungs, shunts.

INTRODUCTION

The black rat, *Rattus rattus* is a host for *Schistosoma mansoni* and plays an important role in the natural transmission of the parasite in the West Indies (Imbert-Establet, 1982; Théron & Pointier, 1995). After intravascular migration of parasites in this host, maturation in the portal system occurs normally, in contrast to the situation in the laboratory rat *R. norvegicus* where self-cure takes place around 28 days post-exposure. However in *R. rattus*, the *S. mansoni* adults subsequently undergo a migration from the porto-mesenteric system to the lungs (Imbert-Establet, 1980). The likely route is via the portal vein, hepatic vein, vena cava and right side of the heart to the pulmonary arteries. This translocation occurs between the 4th and the 20th week post-infection, with a peak between the 4th and the 8th week. It has been demonstrated that the relocation of the adult worms to the lungs of *R. rattus* takes place after the beginning of egg deposition, is correlated with total worm burden and is associated with resistance to re-infection (Imbert-Establet & Combes, 1992). The presence of adult worms in the lungs of mammalian hosts, other than *R. rattus*, has been recorded by other researchers. These studies have involved the 129 strain mouse which presents innate anatomical abnormalities of its hepatic portal vasculature (Coulson & Wilson, 1989; Elsaghier *et al*. 1989), chronically infected mice after curative chemotherapy (Hewitt & Gill, 1962) and, anecdotically, humans (Cheever, 1968*a*).

In the schistosome-infected laboratory mouse injection of microspheres into the superior mesenteric vein and their subsequent fractional distribution between the liver and lung have been used to characterize the development of haemodynamic changes in the portal vasculature (Wilson, Coulson & McHugh, 1983). Additionally, a strong correlation has been detected between the number of parasite eggs in the lungs and the progressive increase in diameter of intra-hepatic porta-systemic connexions together with extra-hepatic collateral vessels (McHugh, Coulson & Wilson, 1987*a*). The presence of these connexions which breach the integrity of the hepatic portal system provides an escape route for both adult worms and migrating challenge schistosomula, thus accounting for the apparent resistance of these mice to reinfection (McHugh, Coulson & Wilson, 1987*b*). Similar results were obtained in *Fasciola hepatica*-infected rats by Ford *et al*. (1987). The phenomenon of shunting was observed from the

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portal system to the systemic circulation. These authors proposed that *F. hepatica*-induced liver damage can contribute to explain heterologous resistance between *F. hepatica* and *S. mansoni*.

The aim of this work was to investigate whether portacaval shunts exist naturally in the vasculature of *R. rattus* (as in the 129/Ola mouse strain) or whether they develop as a consequence of the *S. mansoni* infection, and to relate the translocation of adult worms from the portal system to the lungs, to the occurrence of portacaval shunts.

MATERIALS AND METHODS

Parasite and host

A Guadeloupean strain of *S. mansoni* was maintained in a Guadeloupean strain of *Biomphalaria glabrata*. The *R. rattus* colony originated from Guadeloupe and the individuals used in the experiments were born in the laboratory. The infection technique was adapted from that of Smithers & Terry (1965) and Erickson (1974).

Injection of microspheres

The injection technique was adapted from that used for mice by McHugh *et al*. (1987*a*). A 0·1 ml aliquot of carbonized Polystyrene microspheres (3 Ms Co., Minneapolis), suspended in 10% dextran + Tween 80 (0·5% v/v), was drawn into a 1 ml disposable syringe via a 25-gauge needle. The aliquot contained microspheres with diameters of 8·6 ± S.D. 1·9 μm (9 μm) and 53·2 ± 3·8 μm (50 μm), at an approximate ratio of 9:1. The rats were anaesthetized by an intraperitoneal injection of 0·1 ml of a sodium pentobarbital solution/100 g of body weight. A laparotomy was performed to expose the caecum and intestines, and the aliquot of microspheres injected in the superior mesenteric vein. A piece of absorbable haemostatic sponge (Spongostan, Ferrosan, Denmark), moistened with physiological saline, was placed over the puncture wound in the vein and held in position with a microhaemostat. The hypodermic needle was then withdrawn from the vein with minimal loss of blood. The haemostat was removed after 3–4 min and circulation was seen to resume. The intestines were covered with a moistened swab and the rat left for a further 20–30 min before perfusion.

Perfusion

Worm and microsphere recoveries were performed as follows. The heart and aorta of the anaesthetized rat were exposed and the inferior vena cava was ligatured. The rat was perfused with 100 ml of heparinized NaCl solution (0·9% w/v), 50 ml *via* the aorta and 50 ml *via* the hepatic vein. The perfusate contained parasites and microspheres dislodged from the liver and hepatic portal system. The intestines of each rat were washed 3 times to ensure removal of all the microspheres and parasites. The washings and perfusate were pooled and worms were counted under a binocular microscope. The pooled washings and perfusate were then centrifuged to remove the microspheres from suspension, the supernatant fraction discarded, and the pellet added to the liver sample.

Organ digestion, microsphere and egg counts

In order to determine the fractional distribution of microspheres and eggs in liver and lungs, the organs were digested overnight in 5% KOH at 37 °C (Cheever, 1968*b*). The total numbers of eggs and microspheres present in each organ were estimated from the counts in representative aliquots of the whole suspension.

Statistics

Data were expressed as mean and standard error. In the Man–Whitney test, we compared the proportions of 9 and the proportions of 50 microspheres for each point. The relationships between the parameters were investigated by calculating the correlation coefficients.

Experimental protocol

Microsphere injections and perfusions were carried out on a control group of 4 naive *R. rattus* to provide baseline data.

Experimental bisexual infections. Sixteen *R. rattus* were individually exposed to 1000 cercariae (from pooled multimiracidial-infected *B. glabrata*) for 35 min. Microsphere injections and perfusions were performed on groups of 4, 3, 3, 3 and 3 rats at 5, 7, 8, 10 and 20–25 weeks post-infection, respectively.

Experimental male-unisexual infections. Six *R. rattus* were individually exposed to 1000 cercariae (from pooled *B. glabrata* with male-only *S. mansoni* infections) for 35 min. Microsphere injections and perfusions of these rats were performed at 10 weeks post-infection.

RESULTS

The data obtained from the individual outbred rats were sufficiently homogeneous within groups to provide a clear indication of changes in parasite distribution with time and the impact of the infection on host vascular physiology. We first demonstrated that naive rats lacked portacaval shunts, by injecting

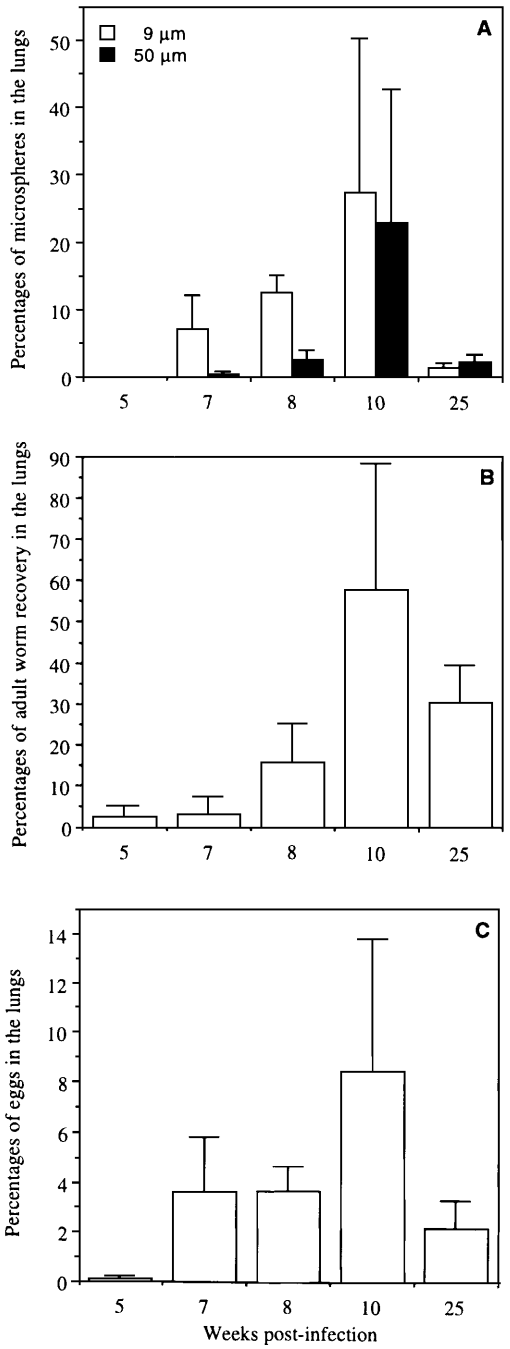

Fig. 1. Percentage recovery of (A) microspheres, (B) adult worms and (C) eggs from the lungs of *Rattus rattus* with time post-infection. All values are mean ± S.E.

microspheres into the superior mesenteric vein. All microspheres were subsequently detected in the portal perfusate and none in the lungs. We then used the same procedure to investigate the development of shunts in rats harbouring a bisexual infection. In these animals egg deposition in the liver had already commenced by week 5 but no microspheres were detected in the lungs at this time (Fig. 1A and Table 1). By week 7 post-infection 7·2% of 9 μm microspheres and 0·5% of 50 μm microspheres were detected in the lungs. By week 8, more microspheres were present in this location but the percentage of

Table 1. Individual percentages of 9 and 50 μm microspheres, adult worms and eggs recovered in the lungs calculated from the total number (WPI: Week post-infection)

		Microspheres			
Rat infection	WPI	9 μm	50 μm	Adult worms	Eggs
Bisexual	5	0·00	0·00	0·41	0·07
	5	0·00	0·00	0·00	0·08
	5	0·00	0·09	10·00	0·41
	5	0·00	0·00	0·00	0·01
	7	0·23	0·38	0·00	0·02
	7	7·12	0·12	0·00	5·17
	7	14·16	0·95	9·92	5·63
	8	11·22	0·27	2·29	2·52
	8	10·11	4·85	29·65	3·22
	8	16·67	2·48	14·94	5·29
	10	0·24	0·35	12·08	0·16
	10	63·45	54·31	97·16	14·92
	10	18·64	14·24	64·71	10·41
	20	2·32	1·04	21·59	1·20
	25	1·32	1·05	45·28	4·03
	25	0·07	4·11	23·56	1·29
Unisexual	10	0·87	0·19	6·33	—
	10	68·44	36·44	97·99	—
	10	13·38	3·39	8·87	—
	10	55·82	70·14	70·59	—
	10	56·78	6·25	4·58	—
	10	42·00	6·25	15·75	—

9 μm microspheres remained significantly higher than for the 50 μm microspheres ($U = 0$; $P = 0{\cdot}05$). At week 10, one rat (with the lowest worm burden) had very few microspheres of either dimension in the lungs. In the remaining 2 rats the upward trend in microsphere shunting continued, with 9 and 50 μm microspheres found in almost equal proportion ($P > 0{\cdot}05$). When rats were sampled at week 20–25, the extent of shunting was greatly diminished with only 1–2% of microspheres detected in the lungs.

Total worm burdens in experimental rats were determined by portal perfusion and dissection of the lungs. There was an apparent downward trend in total mean worm burdens over the course of the experiment (410, 356, 206, 216, and 151 at weeks 5, 7, 8, 10 and 25, respectively). Fig. 1B and Table 1 show the adult worms present in the lungs as a percentage of the total worm burden. At week 5 post-infection, 2 of the 4 rats harboured low percentages of adult worms in the lungs, the remaining 2 were negative. At week 7 only 1 of the 3 rats had any worms in the lungs. All rats harboured worms in the lungs at weeks 8 and 10, and at the latter time in 2 rats the majority of the worm population was present in the lungs. At 25 weeks post-infection the proportion of worms in the lungs had approximately halved to 30% of the total remaining.

Table 2. The degree of association for the total data set between the various parameters measured in the lungs of rats with a bisexual infection

(Values given are correlation coefficients and their level of significance: $*P < 0{\cdot}05$, $**P < 0{\cdot}01$, $***P < 0{\cdot}001$.)

	50 μm Spheres	Eggs	Worms
9 μm Spheres	0·7**	0·92***	0·51*
50 μm Spheres		0·75***	0·78***
Eggs			0·65**

The proportion of schistosome eggs present in the lungs can also provide an indication of the extent of portacaval shunting (Fig. 1C and Table 1). The general pattern follows that of microspheres and worms, with negligible numbers at week 5 rising to a peak of 8·5% of the total at week 10 and declining at week 25. Examination of the degree of association between the various parameters in the lungs of rats with a bisexual infection listed in Table 1 revealed that all were significantly correlated (Table 2). The mean total worm burden at 10 weeks in the rats harbouring male-only schistosome infections was $213 \pm$ S.E. 21. The data from these rats also revealed that, in 5 out of 6 animals, significant numbers of 9 and 50 μm microspheres were detected in the lungs, and in all cases adult worms were present (Table 1). Indeed, the values were in the same range as those at week 10 from rats with bisexual infection. The percentage of 50 μm microspheres correlated with the percentage of adult worms in rats with a male-only infection ($r = 0{\cdot}86$; $P < 0{\cdot}05$), whereas the percentage of 9 μm microspheres did not ($P > 0{\cdot}05$).

DISCUSSION

Our study of *S. mansoni* infection in *R. rattus* has revealed a strong association between loss of portal integrity and the translocation of mature worms from the portal system to the lungs. The lack of microspheres in the lungs of naive *R. rattus* after injection into the superior mesenteric vein demonstrates the integrity of the hepatic portal vasculature prior to schistosome infection. This is unlike the situation in the majority of 129/Ola strain mice which have an innate anomaly of the portal vasculature in the form of patent portacaval anastomoses (Coulson & Wilson, 1989). Subsequent to infection of *R. rattus* with *S. mansoni*, the integrity of the portal system is progressively impaired. Thus, microspheres injected into the superior mesenteric vein are detected in increasing numbers in the lungs, between weeks 7 and 10 post-infection. On the whole, the 9 μm microspheres reach the lungs in greater proportions than the 50 μm microspheres, indicating that some of the portacaval connexions have a restricted diameter. In some instances the degree of portacaval shunting is extensive. Whilst microsphere injection provides an instantaneous measure of shunting, the translocation of eggs from the portal system to the lungs occurs over an extended period. Nevertheless, there is an excellent correlation between the percentage of total eggs present in the lungs and the percentage of microspheres detected. Thus in this model, as in the chronically infected mouse (McHugh *et al.* 1987*a*), the accumulation of eggs in the lungs provides a reliable indicator of portacaval shunting.

The presence of adult worms in the lungs of *R. rattus* has previously been described (Imbert-Establet, 1980; Imbert-Establet & Combes, 1992). The observations reported in the current study clearly show that this is not due to an innate anomaly of the vasculature but results from the formation of portacaval shunts during infection. The translocation of adult worms to the lungs is maximal between weeks 7 and 10, coincident with the development and increasing size of the shunts. The low level of microsphere shunting at 20–25 weeks suggests that the portacaval anastomoses have regressed. This is associated with the lowest mean total worm burden and may have resulted from several causes. Potentially these include fewer worms blocking the portal distributaries, a consequential reduction of egg deposition in the liver, and immunomodulation of granuloma formation, all of which would result in reduced portal hypertension (McHugh *et al.* 1987*a*). However, the fact that there was still a large percentage of worms in the lungs at this time suggests that they can survive in this unfavourable site for a significant period after translocation.

The results obtained using *R. rattus* with a male-only schistosome infection show that, in this species, the development of portacaval shunting does not solely depend upon egg deposition in the liver to generate portal hypertension. The size of the anastomoses that develop in this situation is sufficiently large to permit the translocation of most of the adult worm population to the lungs. In mice superinfected with male schistosomes we have observed portal hypertension but did not ascertain whether portacaval anastomoses developed or translocation to the lungs occurred (P. S. Coulson & R. A. Wilson, unpublished data).

We conclude that the characteristics of a *S. mansoni* infection in *R. rattus* are much closer to those in the laboratory mouse, than to those in *Rattus norvegicus*. In this respect, *R. rattus* is a permissive host for *S. mansoni* with worm maturation, egg deposition and subsequent hepatoportal pathology. This model provides another example of the consequences ensuing from the loss of hepatic portal integrity. Our present study shows that this loss results in an apparent reduction in the adult worms recoverable

by portal perfusion ('anti-adult immunity'?) which is the product of worm translocation to the lungs. Our results also explain previous observations that *R. rattus* with a primary infection are resistant to cercarial challenge compared to naive controls (Imbert-Establet & Combes, 1992), most probably due to the leakiness of the portal system for newly arrived schistosomula (Wilson, 1990).

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